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Uptake and controlled release of a dye from thermo-sensitive polymer P (NIPAM-*co*-Vim)

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ABSTRACT

Copolymers of *N*-isopropylacrylamide (PNIPAM) are one of the most promising microgel materials for medical applications, especially as a drug carrier. PNIPAMs present unique properties, such as size variation with changing pH and/or temperature. The results of a study on the uptake and release of a dye (Orange II) by microgels of *N*-isopropylacrylamide copolymer with 1-vinylimidazole (P(NIPAM-co-Vim) are presented. The dye was used as a model low-molecular substance. Hydrodynamic radius (R_h) of P(NIPAM-co-Vim) particles was measured by dynamic light scattering as a function of temperature in two pH environments: acidic and basic. The dye particles contraction was monitored as a function of temperature at pH 4. The measurements of the zeta potential indicated a positive charge of P(NIPAM-co-Vim) particles at pH 4 and a negative one at pH 9. The key experiments were the internalization and the release of the dye. The effectiveness of this process was measured by UV-Vis spectroscopy on the supernatant derived from centrifuged P(NIPAM-co-Vim) suspension. At room temperature the efficiency of trapping of the dye by the microgel at pH 4 was 87%. Changing pH of a sample initially saturated with the dye from 4 to 9 led to a complete release of the trapped dye.

1. Introduction

Poly(*N*-isopropylacrylamide) (PNIPAM) (Fig. 1a) is one of the most frequently studied polymer for biomedical application. The unique feature of PNIPAM is the phase transition between hydrophilic to hydrophobic state at a temperature around 32 °C known as the lower critical solution temperature (LCST). Above this temperature, initially swollen and well hydrated particles, collapse as a consequence of dominating polymer-polymer interaction. As a result of shrinkage and phase transition, bound water is expelled to the surroundings. This effect of changes in the particle state might be used for controlled drug delivery with PNIPAM microgel particles as a carrier because the LCST temperature is close to the temperature of human body. PNIPAM-based particles were successfully used in research tests as carriers for sulfamethoxazole [1], insulin [2], prednisolone acetate [3], chlorhexidine [4], lidocaine [5], amphotericin B [6], trypsin [7], chymotrypsin [8], calcitonin [9], heparin [10], cobalamin [11], indomethacin [12], doxorubicin [13]. PNIPAM in aqueous solutions has a hydrogel structure that mimics the properties of living tissue, mainly because of a high amount of water bound inside particles. Hydrogels exhibit a minimal tendency to adsorb proteins from body fluids due to the low interfacial tension [14] and they are biocompatible in contact with blood, body fluids and other tissues [15]. Also the internalization process of PNIPAM into living cells occurs spontaneously [16]. Genotoxicity and cytotoxicity studies [17,18] were performed indicating no negative effects. The drug loaded into nanoparticles was transported by the cardiovascular system, where the nanoparticles got opsonized by the blood components to such extent that phagocyte system recognized them as their own [19]. In general, particles greater than 200 nm are filtered by spleen. Smaller particles are phagocytized by liver [20].

The ability of PNIPAM to bind water is caused by hydrophilic interactions of water molecules with polar groups (−C═O, −NH) of the amide function (primary bound water). The hydrophobic groups (i.e. CH_3) are surrounded by less tightly bound water particles film which makes the whole particle more hydrophilic [21]. The hydration of hydrogels starts from 10 to 20% of water content and reaches several thousand percent of dry mass [11]. The hydration above LCST is estimated as 20–30% wt-% [22]. The internal structure for PNIPAM

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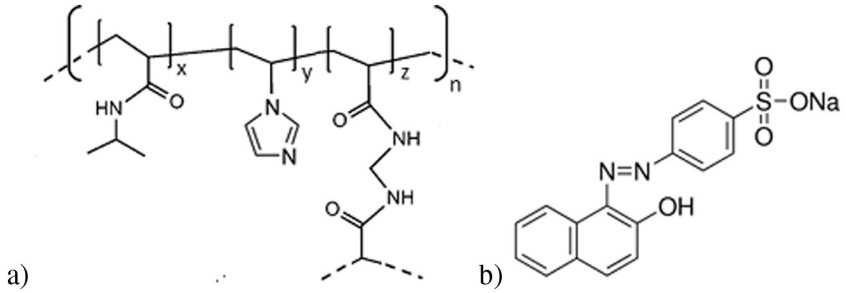

Fig. 1. a) P(NIPAM-co-Vim) crosslinked with BAA, b) Orange II sodium salt.

particles was defined as microgel [23]. The water release process through the particle caused by the temperature change is controlled by two mechanisms [24]. The first mechanism – skin regulated diffusion – is controlled by the weak permeable layer formed on PNIPAM particle surface at temperature above LCST. The second one – squeezing mechanism – is driven by the change in particle volume above the LCST and expelling the water to the surroundings. The diffusion process, alike in the first and in the second mechanism, is controlled by pore size of hydrogel particle structure.

PNIPAM can be modified by introduction of additional functional groups which could improve its ability to bind drugs. A good candidate is vinyl imidazole; its polymer has the ability to bind to various molecules (metal ions, protein structures) by forming complexes [25]. Therefore, some ligands having interaction abilities with the biological molecules (such as dyes or some reactive polymers) may be incorporated more easily into the PNIPAM-based gel structure by using the complexing ability of poly(vinyl imidazole) [26].

In our paper we focused on the possibility of the electrostatic bonding of a model azo dye, Orange II (Fig. 1b), to the isopropyl acrylamide (NIPAM)-1-vinylimidazole (Vim) copolymer P(NIPAM-co-Vim) (Fig. 1.a) slightly cross-linked with bisacrylamide (BAA). Until now only few works describing uptake effectiveness of NIPAM copolymers appeared. The reports describe copolymers of NIPAM with various amounts of Vim which show the ability to complexation of copper ions [27] or aminated poly(1-vinylimidazole) as a new polycation for gene delivery systems [28]. However, to the best of our knowledge, there are no reports concerning release tests. This effort has been undertaken in the present work.

Thus, the aim of our work was to obtain a material having ability to switch the surface charge from positive to negative by changing pH, which will enable the controlled release of physically bonded to the material, electrically charged substances (in our case the model dye). The method described in this work, which utilizes the change of the particle charge to expel the bonded substance has not been yet applied for NIPAM — containing copolymers. The proposed mechanism can be implemented in stimuli responsive materials.

Earlier reports on the uptake and release of Orange II by another NIPAM copolymer [29,30] focused mainly on the effectiveness of this processes at one pH value, not on the physical mechanism. In our work we applied a different approach by detailed description of the bonding process with a potential application to controlled release process in medicine. Thus, the utility of P(NIPAM-co-Vim) as a carrier was tested below and above LCST, at two selected pH values: pH 4 and pH 9 and in the temperature range of 20–40 °C. The tests performed confirmed the ability of the particles to change their size under the influence of temperature at different pH. Additionally, zeta potential measurements gave the information about the particle charge behavior with changing temperature. Finally, the tests of dye bonding and controlled release have been performed.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAM), 1-Vinylimidazole, dodecylethyl dimethyl ammonium bromide (DEDAB), *N*,*N*-methylenbisacrylamide (BIS), 2,2′-azobis(2-amidinopropane)dihydrochloride (V50), 4-(2-Hydroxy-1-naphthylazo)benzenesulfonic acid sodium salt (Orange II) were purchased from Aldrich. Buffers for pH 4 (citrate buffer, ionic strength 0.044, Chempur) and pH 9 (tetraborate buffer, ionic strength 0.075, Chempur). Deionized water (18.6 MΩ·cm) used for synthesis and for dilution was filtered through a 0.2 μm filter.

2.2. Synthesis

Synthesis of NIPAM copolymer with vinylimidazole, P(NIPAM-co-Vim), was performed accordingly to [31,32] in a 0.5 L reaction vessel equipped with a mechanical stirrer, reflux condenser, thermometer, and gas inlet. 3.82 g of NIPAM, 0.10 g of the cross-linker BIS, 0.19 g of 1-Vinylimidazole and 0.17 g of cationic surfactant DEDAB were stirred with 0.3 L of water at 62 °C and purged with nitrogen for at least 1 h. Polymerization was initiated with 0.14 g of cationic initiator V50 dissolved in 5 mL of water and carried out for 6 h under a nitrogen stream at constant stirring at 300 rpm. P(NIPAM-co-Vim) microgel solutions was centrifuged three times for 45 min at 50000 rpm and 25 °C. Between each centrifugation the supernatant was removed and replaced by deionized water to redisperse the microgels. After three cycles of centrifugation the solutions were freeze-dried overnight for storage.

2.3. DSC measurements

The determination of LCST during heating and cooling for P (NIPAM-co-Vim) was based on DSC measurements. The tests were done on water-suspended P(NIPAM-co-Vim) samples. The measurements were performed using Perkin-Elmer instrument DSC 8000. The samples were scanned in temperature range 10–25 °C in two heating-cooling cycles with rate 5 °C/min.

2.4. DLS measurements

The most common, nondestructive method for characterization of colloid particles in water solution is dynamic light scattering (DLS). In the current work, examination of the influence of pH, temperature and presence of Orange II on the change of microparticle size were performed using a DLS system. The system was based on 633 nm He-Ne laser, thermostabilized goniometer with accuracy of 0.1°, avalanche photodiode (SPCM-AQR, Perkin-Elmer) and correlator ALV/5000E (ALV GmbH). Hydrodynamic radii were calculated from the CONTIN [41] analysis of the measured correlation functions. The samples for DLS test were prepared by dispersing of P(NIPAM-co-Vim) in buffer solution of pH 4 and pH 9 at concentration of 0.02 mg/mL. The investigated temperature range was 20–40 °C with a step of 2 °C. Each